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U.S. PATENT AND TRADEMARK OFFICE
PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT
under 37 C.F.R. §1.53(b)(2)

Atty. Docket: KEHOE1

INVENTOR(S)/APPLICANT(S)			
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[ ] Additional inventors are being named on separately numbered sheets attached hereto

TITLE OF THE INVENTION (280 characters max)

THERAPY FOR CANCER

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ENCLOSED APPLICATION PARTS (check all that apply)

[x] Specification Number of Pages 19 [ ]Applicant claims small entity status. See 37 C.F.R. §1.27

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The invention was made by an agency of the United Stated Government or under a contract with an agency of the United States Government.

[X] No [ ] Yes, the name of the U.S. Government agency and the Government contract number are:

_____

Respectfully submitted,

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Date: June 4, 2004

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US PROVISIONAL PATENT APPLICATION KEHOE=1

US PROVISIONAL PATENT APPLICATION

FOR

"Therapy for cancer"

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US PROVISIONAL PATENT APPLICATION KEHOE=1

FIELD OF THE INVENTION

The field of the present invention relates to the use of combined therapies as an anti-cancer therapy. More specifically, the field of the present invention relates to the use of hormonal therapy and immunotherapy as an anti breast cancer therapy.

BACKGROUND OF THE INVENTION

Breast cancer

Breast cancer is the most frequently diagnosed cancer of women in Canada. Over the course of a lifetime, one in nine women is expected to develop breast cancer and one in twenty seven women will die from it. Despite advances in the diagnosis and treatment of breast cancer, up to 50% of newly diagnosed patients may develop metastases. Metastatic breast cancer has a poor prognosis and is generally considered incurable. Typically, the goals of therapy in the setting of metastatic breast cancer are to control the disease and relieve symptoms as much as is possible to maintain or improve quality of life. It has been estimated that the "residual life expectancy" (the additional time a person with cancer would have lived in the absence of the disease) of a woman with breast cancer is 19.6 years. There is a clear and unmet clinical need to develop effective therapeutics for the prolongation of life and relief symptoms for women with metastatic breast cancer.

WO 03/015796 ("Imunogenic conjugate of carbohydrate haptens and aggregated protein carrier") describes an immunotherapy in which an immune response is elicited to a carbohydrate epitope. In particular, an aggregated STn-KLH (heyhole limpet hemocyanin) conjugate, also know as Theratope®, is described.

The Theratope® vaccine developed at Biomira consists of a synthetic STn hapten conjugate to KLH, delivered in emulsion with an adjuvant. The vaccine used in Phase I and Phase II clinical trials had a hapten substitution level that resulted in a sialic acid (NANA) content of about 2.5 to 3% by weight. While Phase II clinical trials were in progress, the conjugation methodology was improved so that a NANA content of about 7$ could be achieved. The high conjugation product induce considerably higher titers of anti-Stn antibody in mice, and significantly higher anti-STn IgG titers in humans in a small bridging study. Since higher anti-STn IgG titers had appeared to be correlated with improved survival in Phase II clinical trials, a large phase II clinical trial was initiated using a STn-KLH conjugate with a NANA content of about 7%

SUMMARY OF THE INVENTION

According to one aspect of there invention there is provided the use of hormonal therapy in combination with immunotherapy (such as Theratope®) as an anti-breast cancer therapy.

According to another aspect of the invention there is provided a kit for the use of hormonal therapy in combination with immunotherapy (such as Theratope®) as an anti-breast cancer therapy.

According to another aspect of the invention there is provided a method to identify patients benefiting from the use of hormonal therapy in combination with immunotherapy (such as Theratope®) as an anti-breast cancer therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

Further details of the invention are described below with the help of examples illustrated in the following drawings, in which:

Figure 1 illustrates patient characteristics;

Figure 2 illustrates overall survival for patients receiving hormone therapy;

Figure 3 illustrates assay results; and

Figure 4 illustrates overall survival and IgG titers in all Theratope patients (T) and patients receiving concurrent hormone therapy (T plus H) or those not receiving hormone therapy (T no H).

EMBODIMENTS OF THE INVENTION

The present invention describes the use of hormonal therapy in combination with immunotherapy (such as Theratope®) as an anti-breast cancer therapy.

Risk factors in breast cancer

There a variety of risk factors that are important in the etiology of breast cancer, these include: increasing age (risk doubles between the ages of 45 and 65), previous breast cancer in the same patient, family history of breast cancer in a first degree relative (mother, sister or daughter), a first degree relative that is pre-menopausal and has bilateral breast cancer, a first degree relative that is pre-menopausal and has uni-lateral breast cancer, and a blood relative that is pre-menopausal and has breast cancer. Additional risk factors include: carriers of mutations of genes such as BRCA1, BRCA2, p53, PTEN, ATM, a family history of cancer of the ovary, cervix, uterus or colon, early menarche, late menopause, nulliparity, first pregnancy over the age of 30, obesity, breast augmentation, oral contraceptives, hormone replacement therapy (HRT), and radiation exposure. Diet and alcohol consumption may also affect the risk of developing breast cancer and this may, in part, explain the fivefold variation in the incidence of breast cancer that is observed among different countries.

Screening and diagnosis of breast cancer

Screening for the presence of breast cancer can be carried out in a variety of way, including: beast self-examination, clinical breast examination, mammography, and screening mammography. Evaluation of abnormalities detected during screening can be carried out by: fine-needle aspiration, ultrasonography, biopsy, mammography,

stereotactic- and ultrasound-guided core biopsies, ultrasound- or stereotactic-guided fine-needle aspiration, magnetic resonance imaging, ultrasound, sestamibi nuclear medicine scanning and positron emission tomography imaging.

Markers of breast cancer

Her-2/neu and c-erB-2 are receptor protein tyrosine kinases that members of the epidermal growth factor receptor (EGFR) family. Over expression of growth factor receptors with homology to EGFR (such as Her-2/neu and/or c-erB-2) have been found to be associated with a poor clinical prognosis of beast carcinoma. It has been noted that those breast carcinomas overexpressing Her2/neu and/or c-erB-2 tend to lack the estrogen and progesterone receptors, and thus are hormone-therapy less-responsive (see below), and have a poor clinical outcome.

Staging of breast cancer

A widely used system to stage breast cancer is the American Joint Committee on Cancer (AJCC) classification, which is based on tumor size (T), the status of regional lymph nodes (N), and the presence of distant metastasis (M), and is referred to as TNM staging. Clinical staging is performed following physical examination and radiologic studies. Pathologic staging is performed following surgery for operable breast cancer (cancer management: a multidisciplinary approach). The stage of the cancer may influence the choice of treatment a skilled clinician may offer to a patient.

Treatment of metastatic breast cancer

Due to the heterogeneity of metastatic breast cancer there are a variety of treatment options for these patients and include, but are not limited to: surgery, chemotherapy, radiation therapy, hormonal therapy and immunotherapy, and/or combinations thereof. The treatment regime will depend on factors such as extent of metastases , comorbid condition and tumour characteristics. Such factors that contribute to the treatment regime are well know to skilled practitioner. Treatment guideline relating to various drug products are also well know to the skilled practitioner, and, by way of example, may be found in the "Compendium of Pharmaceuticals and Specialties (CPS), The Canadian Drug Reference for Health Professionals", and other such guides.

Hormones, breast cancer and hormonal therapy

Hormones, such as estrogen, play an important role in the progression of breast cancer. In premenopausal women, estrogen (17beta-estradiol) is produced predominantly (although not exclusively) in the ovaries through aromatization of estrogen precursors (such as androstenedione) catalyzed by the enzyme estrogen synthetase (aromatase). In menopausal women the ovaries no longer produce estrogen. However, aromatization of adrenal androgen can still occur in peripheral tissues, resulting in the production of 17beta-estradiol. Estrogens are involved in cellular proliferation and the maintenance of breast tissue. The proliferative effects of estrogens are also involved in the promotion of tumor growth in breast cancer, and a number of therapeutic approaches designed to reduce the amounts of estrogen have been developed. In premenopausal women, removal of the ovaries (by oophorectomy, radiation therapy, or biochemical castration) reduces the amount of estrogen and thereby reduces the proliferative effects of estrogen on

tumour growth. In women without functional ovaries, antiestrogens and aromatase inhibitors can be used to reduce the amount of estrogen that is produced in the peripheral tissues. Treatment with antiestrogens and/or aromatase inhibitors can also be referred to as hormonal-therapy or antihormonal-therapy. However, hormonal-therapy and antihormonal-therapy should not be confused with "hormone replacement therapy" (HRT) which is a controversial medical treatment for women with symptoms (hot flashes, night sweats etc.) associated with menopause.

Antiestrogens are used as a therapy in the treatment of metastatic breast cancer and inhibit estrogen-induced proliferation through interaction with the estrogen receptor. Although not all mechanisms of inhibition of cell proliferation have been elucidated, it has been suggested that antiestrogen/estrogen-receptor complex reduces or eliminates the induction of transcription of genes that are under control of the estrogen-response elements. As a result of this reduced gene transcription, cell division is also reduced. Examples of non-steroidal anitestrogens include toremifene, tamoxifen, droloxifene and trioxifene, an example of a steroidal antiestrogen includes fluvestrant.

Aromatase inhibitors reduce the levels of estrogens by inhibiting the aromatase enzyme complex, which is responsible for synthesizing estrogen. Examples of aromatase inhibitors include aminoglutethimide, anastrozole, vorozole, letrozole, formestane, liarozole, exemestane and formestane.

Progestins (such as progesterone) are another type of hormone that play a role in the normal development of breast tissue, control of cell growth and differentiation. Further more, both progestins and antiprogesterines have been used in the treatment of breast cancer.

There are, of course, other compounds whose mechanisms of action as less well elucidated, yet can be considered hormonal therapy. Such compounds can include geoselin acetate (Zoladex®) and megestrol acetate (Megase®)

Chemotherapy

Anthracyclines (for example: doxorubicin, daunorubicin, epirubicin, idarubicin) may be used in the treatment of metastatic breast cancer. Several mechanisms of action may play a role in the anti-tumour effects of anthracyclins, and include: intercalation of DNA, interaction with topoisomerase II, causing strand breaks (single and double) in DNA and formation of free radicals.

Taxanes (for example: paclitaxel, docetaxel) may also be used in the treatment of metastatic breast cancer. Taxanes are antimicrotubular compounds that inhibit cell division by binding to tubulin and inhibiting microtubular disassembly that is required for cell division.

Immunotherapy

The Immune System.

The ability of vertebrates to protect themselves against infectious microbes, toxins, viruses, or other foreign macromolecules is referred to as immunity. The art distinguishes between natural, and acquired or specific immunity (Abbas, et al., Cellular and Molecular Immunology, W. B. Saunders Company, 1991; Hood, et al., Immunology, 2nd Edition, The Benjamin/Cummings Publishing Company Inc., 1984).

Natural immunity is comprised of defense mechanisms which are active before exposure to microbes or foreign macromolecules, are not enhanced by such exposure, and do not distinguish among most substances foreign to the body. Effectors of natural immunity are physical barriers such as skin or mucous membranes, phagocytic cells such as macrophages or neutrophils, a class of lymphocytes termed natural killer cells, and the complement system. Complement is a serum protein complex that is destructive to certain bacterial and other cells sensitized by specific, complement-fixing antibodies; its activity is effected by a series of interactions resulting in proteolytic cleavages and which can follow one or the other of at least two pathways (Illustrated Stedman's Medical Dictionary, 24th Edition, Williams and Wilkins, Baltimore/London, 1982).

Acquired or specific immunity comprises defense mechanisms which are induced or stimulated by exposure to foreign substances.

In vertebrates, the mechanisms of natural and specific immunity cooperate within a system of host defenses, the immune system, to eliminate foreign invaders. In addition to microbes, cancer cells, parasites and virus-infected cells, the immune system also recognizes and eliminates cells or tissues transplanted into a subject from a genetically different individual of the same species (allografts) or from a different species (xenografts).

The events by which the mechanisms of specific immunity become engaged in the defense against invading microorganisms cancer cells, etc. are termed immune responses. Vertebrates have two basic immune responses: humoral and cellular. Humoral immunity is provided by B lymphocytes, which, after proliferation and differentiation, produce antibodies which circulate in the blood and lymphatic fluid. Cellular immunity is provided by the T cells of the lymphatic system. The cellular immune response is particularly effective against fungi, parasites, intracellular viral infections, cancer cells and foreign matter, whereas the humoral response primarily defends against the extracellular phases of bacterial and viral infections.

An "antigen" is a foreign substance which is recognized (specifically bound) by an antibody or a T-cell receptor, regardless of whether it can induce an immune response. Foreign substances inducing specific immunity are termed "immunizing antigens", or "immunogens". An "hapten" is an antigen which cannot, by itself, elicit an immune response (though a conjugate of several molecules of the hapten, or of the hapten to a macromolecular carrier, might do so). Since the present application is concerned with

eliciting immune response, the term "antigen" will refer to immunizing antigens unless otherwise stated.

Tumor Associated Carbohydrate Antigenic Determinants.

Numerous antigens of clinical significance bear carbohydrate determinants. One group of such antigens comprises the tumor-associated mucins (Roussel, et al., Biochimie 70, 1471, 1988).

Generally, mucins are glycoproteins found in saliva, gastric juices, etc., that form viscous solutions and act as lubricants or protectants on external and,internal surfaces of the body. Mucins are typically of high molecular weight (often > 1,000,000 Dalton) and extensively glycosylated. The glycan chains of mucins are O-linked (to serine or threonine residues) and may amount to more than 80% of the molecular mass of the glycoprotein. Mucins are produced by ductal epithelial cells and by tumors of the same origin, and may be secreted, or cell-bound as integral membrane proteins (Burchell, et al., Cancer Res., 47, 5476, 1987; Jerome, et al., Cancer Res., 51, 2908f 1991).

Cancerous tissues produce aberrant mucins which are known to be relatively less glycosylated than their normal counter parts (Hull, et al., Cancer Commun., 1, 261, 1989). Due to functional alterations of the protein glycosylation machinery in cancer cells, tumor-associated mucins typically contain short, incomplete glycans. Thus, while the normal mucin associated with human milk fat globules consists primarily of the tetrasaccharide glycan, gal beta1-4 glcNAcpl-(gal beta1-3) gal NAc-alpha and its sialylated analogs (Hull, et al.), the tumor-associated Tn hapten consists only of the monosaccharide residue, alpha-2-acetamid-2-deoxy-D-galactopyranosyl, and the T-hapten of the disaccharide beta-D-galactopyranosyl-(1-3)alpha-acetamido-2-deoxy-D-galactopyranosyl. Other haptens of tumor-associated mucins, such as the sialyl-Tn and the sialyl-(2-6)T haptens, arise from the attachment of terminal sialyl residues to the short Tn and T glycans (Hanisch, et al., Biol. Chem. Hoppe-Seyler. 370, 21, 1989; Hakomori, Adv. Cancer Res., 52:257, 1989; Torben, et al., Int. J. Cancer, 45 666, 1980; Samuel, et al., Cancer Res., 50, 4801-1990).

The T and Tn antigens (Springer, Science, 224, 1198, 1984) are found in immunoreactive form on the external surface membranes of most primary carcinoma cells and their metastases (>90% of all human carcinomas). As cancer markers, T and Tn permit early immunohistochemical detection and prognostication of the invasiveness of some carcinomas (Springer). The presence of the sialyl-Tn hapten on tumor tissue has been identified as an unfavorable prognostic parameter (Itzkowitz, et al. Cancer, 66, 1960, 1990; Yonezawa, et al., Am. J. Clin. Pathol., 98 167, 1992). Several types of tumor-associated carbohydrate antigens are highly expressed in common human cancers. The Tn, T and STn haptens occur as mucin-type (0-linked) carbohydrate. Additionally, cancer-associated glycosphingolipids such as GM2 and GD3 are expressed on a variety of human cancers.

The altered glycan determinants displayed by the cancer associated mucins are recognized as non-self or foreign by the patient's immune system (Springer). Indeed, in

most patients, a strong autoimmune response to the T hapten is observed. These responses can readily be measured, and they permit the detection of carcinomas with greater sensitivity and specificity, earlier than has previously been possible. Finally, the extent of expression of T and Tn often correlates with the degree of differentiation of carcinomas (Springer).

Carbohydrate-Protein Conjugates. Because the tumor-associated antigens are useful in diagnosis and monitoring of many types of carcinomas, and may also be useful in treatment, many workers have synthesized glycosides of the carbohydrate haptens and of their sialylated analogs and have used these glycosides to conjugate the haptens to proteins or synthetic peptide carriers. The glycosides have generally included an aglycon moiety from which a highly reactive functionality can be generated without altering the saccharide portion of the respective hapten glycoside. The "activated" hapten glycosides are then reacted with amino groups of the proteins or synthetic peptide carriers to form amide of Schiff base linkages. The Schiff base grouping can be stabilized by reduction with a borohydride to form secondary amine linkages; the whole coupling process is then referred to as reductive amination. (Gray, Arch. Biochem. Biophys., 163, 426, 1974) .

For examples of these conjugates, see Lemieux, et at., USP 4,866,045; Naicker, et al., USP 4,935,503; Kolar, USP 4,42_ 284; Feizi, USP 4,563,445; Koganty, USP 5,055,562; Jennings, USP 4,356,170; Roy, USP 5,034,516.

Wong, USP 6,013,779 discloses a method for the formation of conjugates of a carbohydrate hapten to a protein carrier. First, an alpha-olefinic glycoside is prepared by a Fisher-type glycosylation of an olefinic alcohol, such as crotyl alcohol. The alpha-oelfinic glycoside is ozonolyzed to yield the hapten aldehyde (and a second aldehyde as a byproduct). The byproduct is preferably acetaldehyde or a higher aldehyde, not formaldehyde.

The hapten aldehyde is then conjugated to the carrier protein, such as KLH.

STN-KLH Conjugates

The Theratope® vaccine developed at Biomira consists of a synthetic STn hapten conjugated to KLH, delivered in emulsion with an adjuvant. The vaccine used in Phase I and Phase II clinical trials had a hapten substitution level that resulted in a sialic acid (NANA) content of about 2.5 to 3% by weight. While Phase II clinical trials were in progress, the conjugation methodology was improved so that a NANA content of about 7% could be achieved. The high conjugation product induced considerably higher titers of anti-STn antibody in mice, and significantly higher anti-STn IgG titers in humans in a small bridging study. Since higher anti-STn IgG titers had appeared to be correlated with improved survival in Phase II clinical trials, a large Phase III clinical trial was initiated using a STn-KLH conjugate with a NANA content of about 7%.

Epitope Clusters

Carbohydrate epitope clusters have been reported in the literature, but the significance of these have not yet been clearly defined. See Reddish, et al.,

Glycoconjugate J., 14:549-60 (1997) (clustered STn), Ragapathi, et al. Cancer Immunol. Immunother. 48:1-8 (1999). Likewise, clusters of O-glycosylation sites have been reported. See Gendler, et al., J. Biol. Chem., 263:12820 (1988).

We investigated the "STn cluster" content of various STn-KLH formulations, using a mAb that had specificity for clusters and another that reacted with either monomer or clusters. All vaccine preparations had detectable cluster content, but the upper limit of hapten detection was low, probably because of steric hindrance of the mAbs which are much larger than the haptens. Improved potency could not be specifically assigned to the production of spatial arrays (clusters) at higher substitution levels because the cluster density was too high to measure. In the course of these studies, we observed that by making certain changes in process variables as described below, the NANA content of the vaccine can be increased to 7% with little improvement in potency. This suggests that the observed potency improvement in the system hereafter described can not be attributed merely to the formation of hapten "clusters".

See also Sloan-Kettering, W098/46246; Sloan-Kettering, W097/34921; Sloan-Kettering, W099/15201 (fucosyl GM1-KLH conjugate; KLH said to have MW of $5x10^6$) ; Kjeldsen, USP 5,660,834; Zhang, et al., Cancer Res. 55:3364-8 (August 1, 1995); Swiss Prot P04253; Swiss-Prot P80888; Swiss-Prot P02768; Kjeldsen, USP 5,747,048.

The following U.S. patents use the phrase "clustered epitopes": 6,376,463; 6,258,937; 6,180,371; 5,929,220; 5,888,974; 5;859;204; 5,744,446. The following U.S. patents recited "clustered" and "carbohydrate epitopes: 6,365,124; 6,287,574; 6, 013,779; 5,965,544; 5,268,364; 4,837,306.

Cyclophosphamide

Cyclophosphamide (N, N-bis[2-cholorethyl]tetrahydro-2H-1,3,2-oxazaphosphorine-2-amine-2-oxide), a nitrogen mustard derivative, is a cytotoxic agent which causes cross-linking of DNA. It is most effective against rapidly dividing cells, hence its use in cancer chemotherapy. Since it also destroys lymphocyte cells, it is also useful as a immunosuppressive agent, indeed, it is one of the most potent immunodepressants known.

Although most chemotherapeutic agents suppress host immunity, it has been demonstrated that certain chemotherapeutic agents, under specific conditions, are able to augment host anti-tumor immunity. Berd and Mastrangelo, Cancer Res., 48: 1671-75 (1988); Mastrangelo, et al., seminars in Oncology, 13: 186-94 (1986). Campbell, et al., J. -Immunol., 141: 3227 (November 1, 1988) reported that cyclophosphamide reduced the tumor burden in mice implanted with a murine B cell lymphoma, rendering the tumor more amenable to active specific immunotherapy with an anti-idiotype antibody vaccine. Nothing in the article suggests that the idiotype resembled any carbohydrate epitope of the lymphoma. No immunosuppressive mucins are known to be associated with lymphomas. See also Reissman,, et al.,, Cancer Immunol. Immunotherap., 28: 179-84 (1989) (leukemias).

Mitchell, et al., Cancer Res., 48: 5883 (October 15, 1988) treated melanoma patients with cyclophosphamide and, several days later, immunized them with a melanoma cell lysate. The value of cyclophosphamide pretreatment was unclear. While cyclophosphamide seemed to favor increases in circulating cytolytic lymphocyte precursors, it had no effect on concanavalin A-inducible suppressor T-cell levels, and "the patients who received cyclophosphamide here had no better frequency of clinical response than those given the lysate mixture alone." In any event, no immunosuppressive mucins are known to be associated with melanomas.

In some cancers, the tumors themselves seem to release immunosuppressive factors. The most striking example of this phenomenon is Hodgkin's disease, in which a small tumor in a single lymph node releases or induces the release of immunosuppressive factors that have a powerful effect on the entire cell-mediated immune system. Patients with Hodgkin's disease have a poor delayed hypersensitivity response and are abnormally sensitive to intracellular parasitic infections such as tuberculosis and herpes virus infections. Jessup, et al., Cancer Res., 48: 1689 (1988) mentions that when lymphocytes from patients with colorectal carcinoma are incubated with carcinoembryonic antigen (CEA), a factor is secreted that inhibits immune responses. It has not been recognized previously, however, that tumor immunosuppressive activity can be mediated by mucins. It has been reported that TA3Ha cells are immunoresistant; this is not the same as immunosuppressive.

The present invention is not limited to the use of any particular adjuvant. Other chemical and microbial adjuvants, such as CFA, SAF-1, MDPI BCG, liposomes, and Bordetella pertussis toxin may be used in place of Ribi. The tumor-associated hapten may be conjugated to other carrier proteins, such as tetanus or diphtheria toxoid, or retrovirus peptides (e.g., VP6 viral peptide),, rather than KLH, and the hapten/molecule-to-carrier molecule substitution ratio may be varied. Either natural or synthetic antigens which cross-react with the immunosuppressive mucin may be employed.

The time interval between administration of the cyclophosphamide and administration of the synthetic tumor-associated glycoconjugate is not fixed, but is dependent on the time of onset and duration of action of the cyclophosphamide's inhibitory effect on suppressor T cell activity or on the induction of such activity by tumor-expressed mucins. The dosage of cyclophosphamide may be selected to increase the antigenic specificity of the anti-suppressor T cell activity effect.

In place of cyclophosphamide, another antagonist of immunosuppression may be employed,, such as other oxazaphosphorines, cimetidine or an anti-(suppressor cell) or anti-(suppressor factor) monoclonal antibody. Numerous antibodies of these two types are offered for sale (see Linscott's Directory of Immunological and Biological Reagents, p. 10, 5th ed., 1988,89).

The present invention is not to be restricted on the basis of the present interpretation of the mechanism whereby cyclophosphamide or a similar agent exercises an immunopotentiating effect. An agent antagonizes the immunosuppressive effect of a

tumor-associated mucin if it interacts with the mucin or the T cell so that the mucin no longer activates suppressor T cell activity, or if it interacts with a T cell so activated or its suppressor factors so as to diminish the suppressor activity induced by said mucin, or if it interacts with other components of the cellular immune system so as to render them less vulnerable to suppressor T cells activated by said mucin or to suppressor factors released by such cells, in another embodiment, a monoclonal antibody specific for an epitope of a tumor-associated, immunosuppressive mucin is attached to a suitable support to form an immunosorbent. Circulating tumor-associable immunosuppressive mucins recognized by the immunosorbent are removed from the patient's bloodstream by plasmapheresis. The immune response to the tumor, with or without further stimulating the immune system by active specific tumor immunotherapy, is thereby enhanced. (Lectins or other binding substances might be-used in place of antibodies).

The present invention relates to the novel method of treating metastatic breast cancer combining hormonal therapy and an immunogenic conjugate of a plurality of carbohydrate haptens to an aggregated multimeric protein carrier.

The aggregation results from the interaction of individual monomers of the protein carrier to form a multimeric entity. The interaction may be through binding, and/or through entanglement of the individual protein chains (before, during or after attachment of the carbohydrate haptens). If binding contributes to the oligomerization, it may be covalent and/or noncovalent.

Preferably, the aggregation occurs more or less simultaneously with the attachment of the carbohydrate haptens to the protein.

It is believed that the immunogenic potency of these preparations is attributable to the combination of a high hapten substitution ratio, and the aggregation of the protein carriers to form multimeric entities.

The multimeric entities preferably are dimers, trimer, tetramers, and/or pentamers of the monomeric unit of the protein carrier.

Carbohydrate Haptens; Epitopes

The carbohydrate hapten of the present invention is a carbohydrate which comprises (and preferably is identical to a carbohydrate epitope.

The term "carbohydrate" includes monosaccharides, oligosaccharides and polysaccharides, as well as substances derived from the monosaccharides by reduction of the caronyl group (alditols), by oxidation of one or more terminal groups to carboxylicacids, or by replacement of one or more hydroxy groups by a hydrogen atom, an amino group, a thiol group, or similar heteroatomic groups. It also include derivatives of the foregoing

Normally, a carbohydrate hapten will not be a polysaccharide, as a polysaccharide is usually large enough to be immunogenic in its own right. The borderline between an oligosaccharide and a polysaccharide is not fixed, however, we will define an oligosaccharide as consisting of 2 to 20 monosaccharide (sugar) units.

The hapten may be a monosaccharide (without glyosidic connection to another such unit) or an oligosaccharide. If an oligosaccharide, it preferably is not more than 10 sugar units.

Monosaccharides are polyhydroxy aldehydes ($H[CHOH]_n$-CHO) or polyhydroxy ketones (H-$[CHOH]_n$-CO-$[CHOH]_m$-H) with three or more carbon atoms.

Each monosaccharide unit may be an aldose (having an aldehydic carbonyl or potential aldehydic carbonyl group) or a ketose (having a ketonic carbonyl or potential ketonic carbonyl group). The monosaccharide unit further may have more than one carbonyl (or potential carbonyl) group, and hence may be a dialdose, diketose, or aldoketose. The term "potential aldehydic carbonyl group" refers to the hemiacetal group arising from ring closure, and the ketonic counterpart (the hemiketal structure).

The monosaccharide unit may be a cyclic hemiacetal or hemiketal. Cyclic forms with a three membered ring are oxiroses; with four, oxetoses, with five, furanoses; with six, pyranoses; with seven, septanoses, with eight, octaviruses, and so forth. The locants of the positions of ring closure may vary.

The monosaccharide unit may further be a deoxy sugar (alcoholic hydroxy group replaced by hydrogen), amino sugar (alcoholic hydroxy group replaced by amino group), a thiosugar (alcoholic hydroxy group replaced by thiol, or C=) replaced by C=S, or a ring oxygen of cyclic form replaced by sulfur), a seleno sugar, a telluro sugar, a (-substituted monosaccharide, an unsaturated monosaccharide, an aza sugar (ring carbon replaced by nitrogen), an amino sugar (ring oxygen replaced by nitrogen) an alditole (carbonyl group replaced with CHOH group), aldonic acid (aldehydic group replaced by carboxy group), a ketoaldonic acid, a uronic acid, an aldaric acid, and so forth.

Sialic acid, also known as N-acetyl neuraminic acid (NANA), is of particular interest. It is the terminal sugar on several tumor-associated carbohydrate epitopes.

Tumor associated carbohydrate epitopes are of particular interest.

A variety of carbohydrates can be conjugated according to the present invention, for use particularly in detecting and treating tumors. The Tn, T, sialyl Tn and sialyl (2->6)T haptens are particularly preferred.

In particular, for detecting and treating tumors, the three types of tumor-associated carbohydrate epitopes which are highly expressed in common human cancers are conjugated to aminated compounds. These particularly include the lacto series type 1 and type 2 chain, cancer associated ganglio chains, and neutral glycosphingolipids.

The immunotherapeutic compound of this invention can be made according to International Application Number PCT/US02/24735 (International Publication Number WO03015796). See also International Application number PCT/US90/01856 (International Publication Number WO9011764).

EXAMPLE 1

Background: Theratope is an investigational therapeutic cancer vaccine consisting of a synthetic form of the tumor associated antigen Sialyl Tn (STn) conjugated to the carrier protein keyhole limpet hemocyanin (KLH). While several phase I and II Theratope clinical trials have been conducted, this reports the preliminary results of the phase III clinical trial. **Patients and Methods:** Metastatic breast cancer patients (MBC pts) who had no evidence of disease (NED) or non-progressive disease (NPD) following any first-line chemotherapy were randomized 1:1 to receive adjuvant plus Theratope or control [adjuvant plus KLH]. All patients received a single, low-dose, IV infusion of cyclophosphamide before vaccine. Primary endpoints were time to disease progression (TDP) and overall survival (OS). Pts were stratified by disease status and concomitant hormone therapy (HT). **Results:** 1028 patients were randomized: 32% received concurrent HT and 89% had NPD. Median TDP and OS are shown for Theratope (T) and control (C) pts in the ITT population and pre-stratified hormone groups (table).

Population	ITT		HT and NPD		No HT and NPD	
	T	C	T	C	T	C
N	523	505	152	143	315	306
Median TDP mo	3.4	3.0	8.1	5.6	2.8	2.8
(p value)	(0.35)		(0.42)		(0.48)	
Median OS mo	23.1	22.3	34.3	30.1	18.9	19.0
(p value)	(0.92)		(0.17)		(0.60)	

Theratope was generally well tolerated. Injection site ulceration occurred with similar frequency in the Theratope and control groups (17.5% vs. 17.6%); 8% of patients discontinued due to adverse events. **Conclusions:** In the ITT population, Theratope did not influence TDP or OS. Surprisingly, trends to improve TDP and OS were noted in patients receiving hormonal-therapy (HT) and Theratope compared with HT and control. Since peak immune responses were previously seen at 17 wks in phase II studies, this differential effect may result from an inability to attain optimal immune responses in the non-HT MBC pts due to rapid TDP (<12 wks). Note, "N" in the table denotes the number of patients.

Possible mechanisms for the synergistic action of hormone and immunotherapy treatment of cancer patients

The finding that patients that have undergone or are undergoing hormone treatment (e.g. treatment with estrogen receptor inhibitors or aromatase inhibitors) are more susceptible to immune therapy than cancer patients that have not received such treatment, is surprising and the mechanism of action is not, at this point, clear. Examples of possible mechanisms are however:

1) ***The hormone treatment has affected the specific cellular marker that serves as antigenic target for the targeted immunotherapy, thereby increasing the number of targets for specific immunotherapy.***

Both the expression of protein targets and carbohydrate epitopes has been shown to be affected by hormonal status. Thus, it has been suggested in the literature that expression of muc1 on adenocarcinoma cells is modulated by estradiol and tamoxifen (Pazkiewicz-Gadek et al, Gynecol Endocrinol 2003 Feb 17(1):37-44. There is also evidence that the hormone status of pregnant women alters the expression of carbohydrate epitopes (e.g. Kovalevskaya et al, J. Endocrinol. 2002 Mar; 172(3):497-506) and glycosyl transferases (e.g. Lacord-Bonneau et al, Int. J. Biochem., 1988;20(9):997-1000.

2) ***The hormone treatment has altered the immune status of the patient, thus rendering the individual more responsive to immune therapy in general.***

There is a substantial body of evidence supporting the view that estrogen and hormone therapy can affect the immune status of a host (e.g. Jarvinen et al, Scand. J. Immunol., 1996 Jul;44(1):15-20 and Haeryfar et al, 2000, May-Jun;20(3A):1849-53.

3) ***The hormone treatment affects the cancer cells in such a way that they are rendered more susceptible to immune therapy. This refers to a mechanism that is not primarily relying on the plain expression of antigenic target.***

A number of mechanisms have been implied in the anticancer action of hormone therapies. In addition to its action through estrogen/progesterone receptors (which includes involvement of e.g. PKA and Sp1 in the induction of p27), hormone therapies such as tamoxifen has been shown to affect cancer cells through other mechanisms of action. An example of such a mechanism is its effect on transcriptional level of transforming growth factor beta (TGF)-beta isoforms in tumor tissue (Brandt et al, 2003, Jan-Feb;23(1A):223-9). One could also speculate that the general reduction in tumor size or reduced proliferation induced by hormone treatment could render the tumor or micrometastasis more sensitive to attack by the immune system. Alternatively, hormone antagonists could interfere with muc1 signaling in cancer cells, thereby augmenting the effect of immune therapy induced cancer cell killing..

EXAMPLE 2: results partially stemming from updated EXAMPLE 1 data.
Background Theratope® vaccine is an investigational therapeutic cancer vaccine consisting of a synthetic form of STn conjugated to KLH. Phase III study results showed increased survival in metastatic breast cancer (MBC) patients (pts) concurrently treated with Theratope (T) and hormone therapy (HT) (36.5 mo (T) vs. 30.7mo (control, C), Cox P= 0.04). The term concurrently as used herein is understood to mean that the use of HT and Theratope in patients overlaps. That is to say, HT treatment may start before, after or at the same time as, Theratope treatment is initiated. **Methods:** Metastatic breast cancer patients with either no evidence of disease or non-progressive disease following any first-line chemotherapy were eligible for a randomized, controlled, double-blind, multicenter study. Study arms were adjuvant (Enhanzyn) plus Theratope (generally 100μg per administration) or adjuvant plus KLH (control; C). All patients received a single IV dose of cyclophosphamide (generally 300mg/m^2 to a maximum of 600 mg/m^2) 72±24 hours before receiving the vaccine. Primary endpoints were overall survival (OS) and time to progression. Secondary endpoints were humoral (IgG) responses to the KLH (carrier), STn (synthetic monomeric form), and OSM (naturally occurring clustered STn) antigens in MBC pts +/- HT treated with Theratope. IgG was measured using ELISA assays for STn, OSM, and KLH at week-12 of tx. Median OS was prospectively assessed as a function of IgG titers. **Results:** Of a planned accrual of 950 patients, 1030 were enrolled and 1028 randomized to receive adjuvant + Theratope (n=523) or Control (n=505). As of Sept 2003, median duration of follow-up was 22.2 months. Median titer of anti-OSM IgG was 320 in the Theratope group and 0 in the Control group. Median OS was

significantly greater in T treated pts who received concurrent HT and who had anti-OSM IgG titers equal to or higher than the median titer (41.1 vs. 25.4 mo, log-rank p=0.01). OS was not influenced by anti-STn or anti-KLH titers, indicating that only titers of the naturally occurring clustered STn antigen are predictive of increased OS.

Background and objective

Phase I/II studies of Theratope showed that high IgG levels at week 12 specific for clustered-STn (as measured by ovine submaxillary mucin (OSM) anti-OSM response) but not high anti-KLH IgG correlate with favorable survival.

Median anti-OSM IgG titers were 320 at week 12 in a previous study with the current formulation.

Objectives were to:

Measure anti-STn, anti-OSM, and anti-KLH antibody titers and confirm median antibody titers previously measured.

Determine whether overall survival correlates with increased titers of antibodies to the naturally occurring clustered form of STn in MBC patients from the Phase III Theratope clinical trial.

Study Design and Methods

Study Design

• Phase III, randomized, blinded, controlled study in 126 sites worldwide.

• 1028 MBC patients with either no evidence of disease or non-progressive disease following first line chemotherapy were enrolled.

• Subjects were stratified by disease status and concomitant hormone therapy.

• Subjects were randomized 1:1 to receive adjuvant plus Theratope (n= 523) or control (n=505) [adjuvant plus KLH carrier molecule].

• Vaccine schedule: week 0*, 2 ±2 days)*, 5(±2 days)*, 9(±2 days)*, 13(±2 days), 17(±2 days), 21(±2 days), 25(±7 days), then every 3 months (±7 days) to progression (* plus adjuvant).• All subjects received a single IV dose of cyclophosphamide (generally 300mg/m2 to a maximum of 600mg/m^2) 72 ±24 hours before first vaccine administration.

Data analyses

• Anti-STn (synthetic monomeric form), and anti-OSM (naturally occurring clustered STn) antibodies were measured in serum from MBC pts +/- hormone therapy treated with Theratope using ELISA assays at week-12 of vaccine therapy:

• Baseline sera from study subjects was collected

• ELISA assays were specific for:

anti-STn-IgG & anti-STn-IgM (monomeric STn)
anti-OSM-IgG & anti-OSM-IgM (clustered STn)
anti-KLH-IgG & anti-KLH-IgM (carrier molecule)

§ Pooled sera from responding subjects was used as positive control• Median OS was prospectively assessed as a function of IgG titers.

1. Patient Characteristics:

• Median patient age was 53 yr.

• 89% of all patients had non-progressive disease (56% of subjects with partial responses, 23% of subjects with stable disease, and 11% with minor responses) and 11% had no evidence of disease following first line chemotherapy.

• Over 50% of patients tested had estrogen receptor positive tumors.

• 31% of patients received concurrent hormone therapy as illustrated in Figure 1.

2. Overall Survival for Patients Receiving Hormone Therapy is illustrated in Figure 2. (Survival for patients not receiving hormone therapy was not significantly different between Theratope and Control.). .

3. Assay Result are illustrated in Figure 3: 12-wk median titers in all subjects treated with Theratope or control and in subjects stratified by concomitant hormone therapy (H) or no hormone therapy (NH). In previous studies, median titers of anti-OSM IgG were 320 at week 12.

4. Overall Survival and IgG titers in all Theratope patients (T) and patients receiving concurrent hormone therapy (T plus H) or those not receiving hormone therapy (T no H), is illustrated in Figure 4. Median OS was significantly greater in Theratope treated pts who received concurrent hormone therapy and who had IgG titers of anti-OSM equal to or higher than the median titer (41.1 vs. 25.4 mo, log-rank p=0.01). Median survival was not significantly different as a function of anti-STn or KLH titers. See Figure 4.

5. Tolerability Theratope was generally well tolerated. Injection site ulcerations occurred with similar frequency in the Theratope and control groups (17.5% vs. 17.6%); 8% of pts discontinued due to adverse events.

Conclusions

•Median anti-OSM IgG titers in Theratope-treated patients with NED or NPD following 1st line chemotherapy of Stage IV disease were equal to humoral immune responses previously measured.

•Median overall survival was significantly greater in Theratope treated patients who received concurrent hormone therapy and who had IgG titers of anti-OSM equal to or higher than the median titer (41.1 vs. 25.4 mo, log-rank p=0.01).

•Survival was not significantly influenced by anti-STn or anti-KLH titers, indicating that only titers of the naturally occurring clustered STn antigen are predictive of increased OS.

•Experimental vaccine trials suggest that the ability to mount an immune response is associated with favorable clinical outcomes. Our data from this large, prospective trial indicate that the magnitude of the immune response to the relevant and specific antigen is most predictive of a favorable clinical outcome.

These data also suggest a method to determine who may benefit from the use of hormonal therapy in combination with immunotherapy. In this instance, a patient receiving hormonal treatment in combination with Theratope may have samples tested per the method of Figure 4. Those patients with IgG titers of anti-OSM lower than the median titer may not benefit from hormonal/Theratope treatment and should be directed to an alternate treatment, known to the practitioners skilled in the art.

What is claimed:

1. The use of hormonal therapy in combination with immunotherapy (such as Theratope®) as an anti-breast cancer therapy.

2. The use as claimed in claim 1 wherein the immunotherapy is Theratope®.

3 A kit for the use of hormonal therapy in combination with immunotherapy (such as Theratope®) as an anti-breast cancer therapy.

4 A kit as claimed in claim 3 wherein the immunotherapy is Theratope®.

5. A method to determine who will benefit from the use of hormonal therapy in combination with immunotherapy (such as Theratope®) as an anti-breast cancer therapy.

6. A method as claimed in claim 5 wherein the immunotherapy is Theratope®.

FIGURE 1

Patient Characteristics.
31% of patients received concurrent hormone therapy.

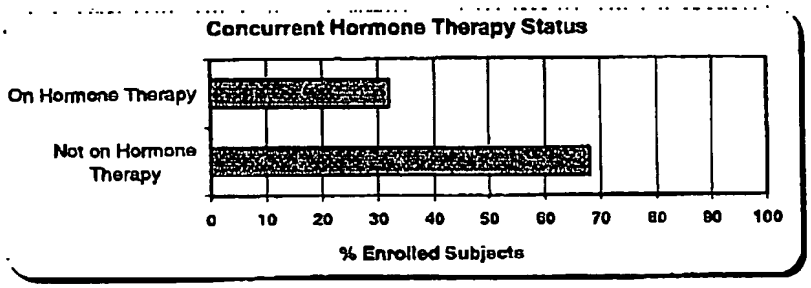

FIGURE 2

Overall Survival for Patients Receiving Hormone Therapy.
(Survival for patients not receiving hormone therapy was not significantly different between Theratope and Control)

			Difference	HR	Cox P value
Theratope	**(n=180)**	36.5 mo	+5.8	1.32	0.04
Control	**(n=170)**	30.7 mo			

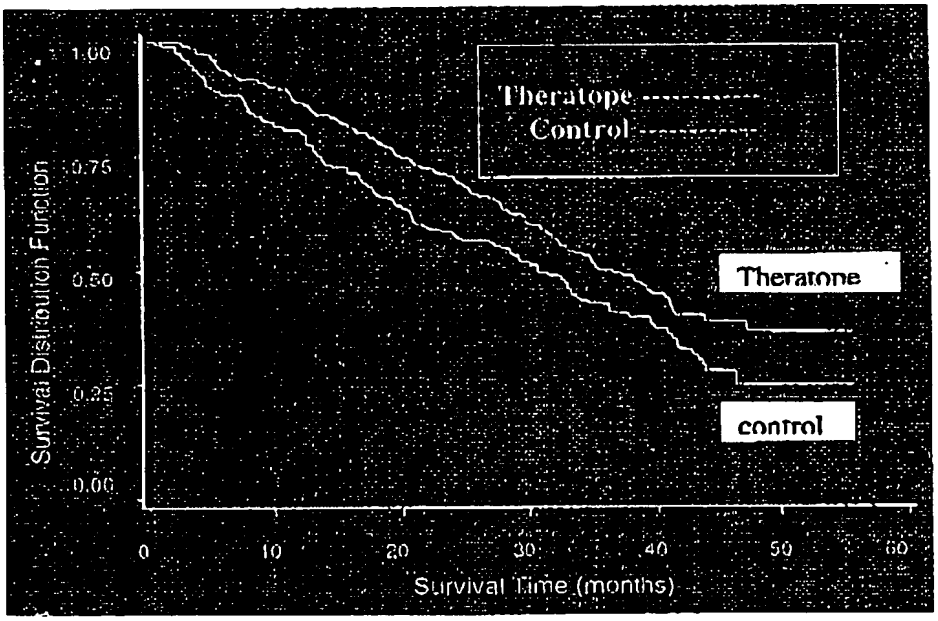

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FIGURE 3

Assay results: 12-week median titers in all subjects treated with Theratope or control and in subjects stratified by concomitant hormone therapy (H) or no hormone therapy (NH). In previous studies, median titers of anti-OSM IgG were 320 at week 12.

Week 12 Median Titers						
	anti-STn-IgG	anti-STn-IgM	anti-OSM-IgG	anti-OSM-IgM	anti-KLH-IgG	anti-KLH-IgM
Theratope	20480	10240	320	1280	20480	80
Theratope H	20480	10240	320	1280	20480	80
Theratope NH	20480	5120	320	1280	20480	160
Control	0	0	0	0	81920	1280
Control H	0	0	0	0	81920	1280
Control NH	0	0	0	0	81920	1280

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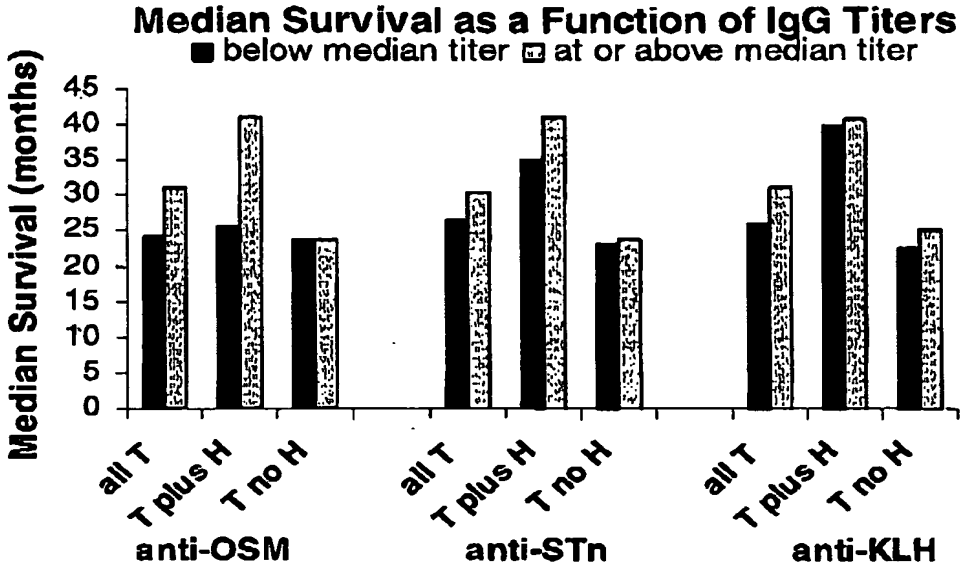
FIGURE 4

Overall Survival and IgG titers in all Theratope patients (T) and patients receiving concurrent hormone therapy (T plus H) or those not receiving hormone therapy (T no H) Median OS was significantly greater in Theratope treated pts who received concurrent hormone therapy and who had IgG titers of anti-OSM equal to or higher than the median titer (41.1 vs. 25.4 mo, log-rank p=0.01). Median survival was not significantly different as a function of anti-STn or KLH titers.



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